Review

Nuno D. Pires*

Seed evolution: parental conflicts in a multi-generational household

Abstract: Seeds are multi-generational structures containing a small embryonic plant enclosed in layers of diverse parental origins. The evolution of seeds was a pinnacle in an evolutionary trend towards a progressive retention of embryos and gametes within parental tissue. This strategy, which dates back to the first land plants, allowed an increased protection and nourishing of the developing embryo. Flowering plants took parental control one step further with the evolution of a biparental endosperm that derives from a second parallel fertilization event. The endosperm directly nourishes the developing embryo and allows not only the maternal genes, but also paternal genes, to play an active role during seed development. The appearance of an endosperm set the conditions for the manifestation of conflicts of interest between maternal and paternal genomes over the allocation of resources to the developing embryos. As a consequence, a dynamic balance was established between maternal and paternal gene dosage in the endosperm, and maintaining a correct balance became essential to ensure a correct seed development. This balance was achieved in part by changes in the genetic constitution of the endosperm and through epigenetic mechanisms that allow a differential expression of alleles depending on their parental origin. This review discusses the evolutionary steps that resulted in the appearance of seeds and endosperm, and the epigenetic and genetic mechanisms that allow a harmonious coinhabitance of multiple generations within a single seed.

Keywords: endosperm; imprinting; MADS; polycomb; triploid block.

***Corresponding author: Nuno D. Pires,** Institute of Plant Biology and Zürich–Basel Plant Science Center, University of Zürich, CH-8008 Zürich, Switzerland, e-mail: nuno.pires@botinst.uzh.ch

Introduction

Seeds were one of the key innovations that allowed gymnosperms and angiosperms to dominate terrestrial ecosystems during the last 300 million years. The protection and nourishment offered to the plant embryos is a costly strategy, but one that greatly increases their chances of survival and dispersion on land. The evolution of seeds followed a trend that started with the first land plants and consisted of the retention of a fertilized zygote and resulting diploid generation within maternal tissue. Subsequent plant lineages elaborated on this strategy to the point of angiosperm seeds forming a multi-generational structure comprising an embryo enveloped in sibling biparental endosperm and two ancestral generations of maternal tissue. The evolution of a biparental endosperm in angiosperms was a particularly important innovation because it allowed fathers to be directly involved in embryogenesis and compete with other parents for resource allocation for their progeny. Importantly, the endosperm also allowed a more sophisticated regulation of the epigenetic development of embryos. The balancing of parental information in the endosperm became an important process in seed development, and underlies an important post-zygotic hybridization barrier in plants. In this review the genetic and epigenetic mechanisms that integrate parental information during seed development will be discussed within the larger context of plant evolution.

Land plant reproduction: a strategy of overprotective parenting

One central and defining characteristic of land plants is the alternation of multicellular generations: a haploid entity (the gametophyte) differentiates gametes, gametes fuse to form a zygote, and the zygote gives rise to a diploid entity (the sporophyte), which forms haploid spores by meiosis. In contrast, in the aquatic ancestors of land plants (the charophyte algae) the fertilized zygote is the only

diploid cell and there is no multicellular spore-producing generation. Even in derived and complex charophytes, such as *Coleochaete* (where egg cells are enveloped and protected by a layer of gametophytic cells) the fertilized zygote directly undergoes meiosis to form free-dispersing haploid spores (1).

One of the crucial innovations of the first land plants was an intercalation of mitotic divisions in the zygote before meiosis, causing the development of a multicellular embryo (a young sporophyte) within gametophytic tissue (2, 3). The sheltered multicellular sporophyte increased the number of spores that could be produced from a single water-dependent fertilization event, offering a huge competitive advantage on land. The rapid increase in the size and complexity of sporophytes that followed led to an explosion of land plant forms in the Devonian period (4), perhaps as soon as 50 million years after the transition to land.

While the sporophytes grew, the gametophytes (which still nourished and protected the embryos) became smaller. Eventually, female gametophytes were themselves retained within parental sporophytic tissue (Figure 1). The oldest evidence for these changes is 385 million year-old fossils of *Runcaria* (5). Later, the evolution of specialized integuments resulted in the appearance of the first true ovules and seeds around 365 million years old (6).

These first seeds (and the seeds of modern gymnosperms) consisted of three distinct genetic generations: 1) a sporophytic embryo, nourished by 2) a female gametophyte enveloped in 3) a maternal sporophytic coat (Figure 1). With the retention of female gametophytes within sporophytic tissues, vascular plants achieved a

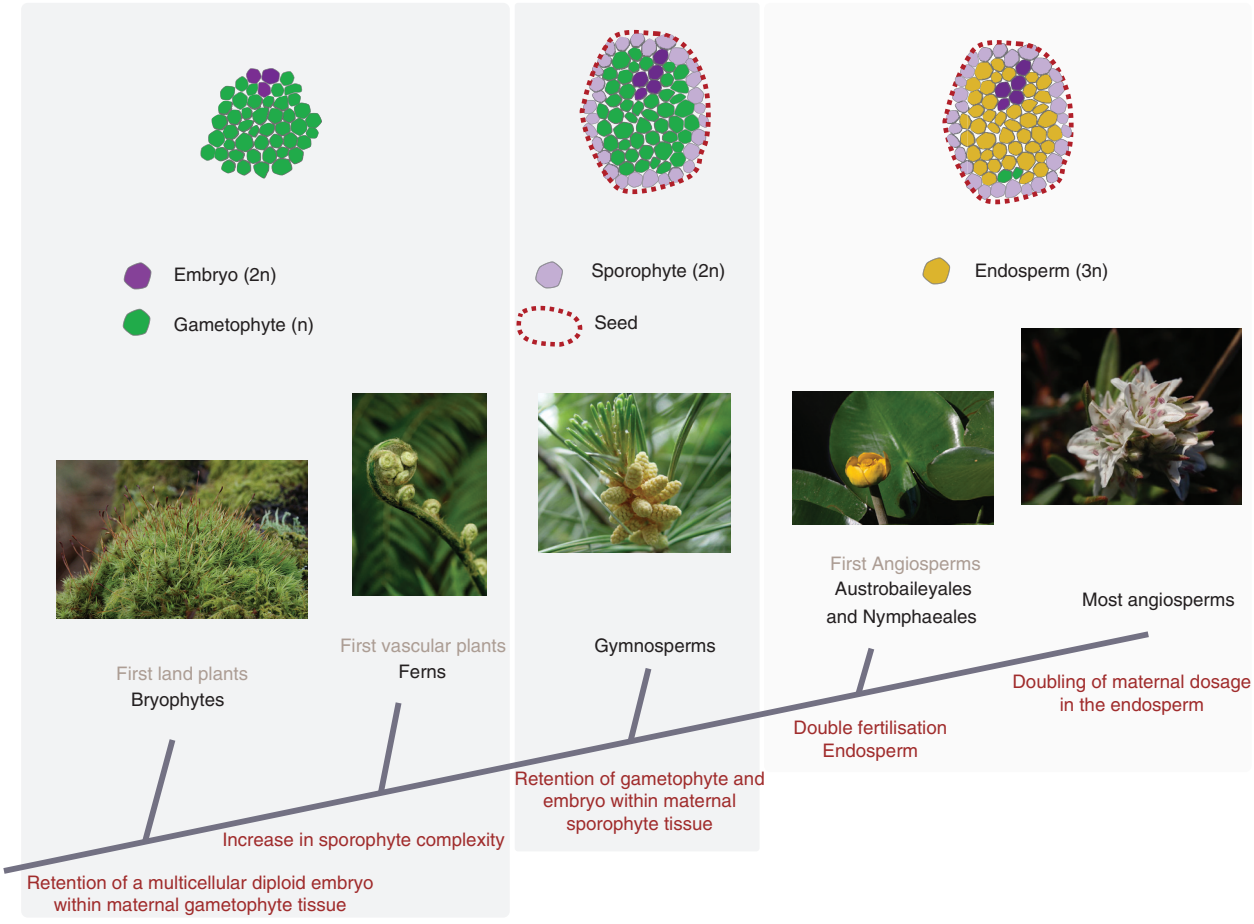

Figure 1 Progressive retention of the diploid embryo within parental tissues during land plant evolution.
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greater independence from water for fertilization, while the production of seeds provided a sophisticated and effective method of dispersal. Seeds are usually very resistant structures that can stay dormant and travel long distances before germination, a process that is supported by nutritional reserves accumulated in the female gametophyte. The success attained by seed-producing plants is attested by the dominance of gymnosperms in the world flora for most of the last 300 million years (7).

The flowering plants

In the Early Cretaceous period (100–145 million years ago), a group of seed plants evolved another set of extremely successful reproductive innovations that made them the dominant plant group in terrestrial environments for the last 100 million years (7). The most obvious innovation of this group – the flowering plants or angiosperms – is the flower, a sophisticated complex of reproductive organs that promotes pollination and fertilization. A second major innovation of flowering plants is that their ovules are harbored in an ovary that develops into a fruit after fertilization, offering another layer of protection to the embryo and greatly increasing the potential for effective seed dispersal. A third more subtle but equally revolutionary innovation was the evolution of the endosperm, a biparental entity that acts as an embryo-nourishing tissue.

The endosperm evolved in parallel with a double fertilization mechanism that is universal in flowering plants. The pollen tube (male gametophyte) releases two sperm cells into the embryo sac (female gametophyte): one sperm cell fertilizes the egg cell to give rise to a zygote, while the second sperm cell fuses with the central cell of the embryo sac to give rise to the endosperm. The endosperm develops together with the sibling embryo, nourishing it and carrying food reserves that are often used later during seed germination.

The emergence of a biparental nourishing endosperm in the seeds of flowering plants is a pinnacle of an evolutionary trend towards increased parental control over plant embryo development. Developing angiosperm seeds are complex multi-generational structures that contain 1) a sporophytic embryo embedded in 2) sibling endosperm, 3) the receding female gametophyte [which can persist during early seed development in a few species (8)] and 4) a maternal sporophytic seed coat (Figure 1).

With the evolution of double fertilization, the control over embryogenesis shifted from the female gametophyte to the biparental endosperm, breaking maternal hegemony over the control of embryogenesis. The intrusion of fathers on the control of the embryogenesis process set the scene for potential conflicts between maternal and paternal genomes over the allocation of resources during embryogenesis. The clash of paternal, maternal and offspring interests that ensued will be discussed later in this review.

The origins of double fertilization and the endosperm

The evolution of flowering plants in the Cretaceous period was an extremely dynamic and innovative time in the history of eukaryotic life. Reconstructing the evolutionary steps that resulted in the appearance of flowers and the radiation of angiosperms has been a puzzle for botanists since the time of Darwin ('an abominable mystery'). Tracing back the evolutionary origins of the endosperm has similarly been an arduous task since the discovery of double fertilization at the end of the 19th century (9, 10).

Two competing hypotheses were soon advanced to explain the origin of the endosperm. It was proposed to be either homolog to a supernumerary embryo that fails to develop into a plant (11) or homolog with the female gametophyte (12, 13, reviewed in 14). This discussion went on through the 20th century, and until today the mechanisms that drove the evolution of double fertilization are not fully understood. Surprisingly, the Gnetales (a derived lineage of gymnosperms) were found to also undergo a well-defined double fertilization event, where a second sperm cell nucleus fuses with a sister egg cell nucleus, giving rise to a second embryo (15, 16). The discussion over the phylogenetic positioning of the Gnetales (nested within other gymnosperms vs. sister to the flowering plants) is not yet closed (17). Nevertheless, the presence of a double fertilization mechanism in the Gnetales suggests that the ancestors of flowering plants (or indeed the first seed plants) were experimenting with different types of multiple fertilization, and that a pro-endosperm could have potentially evolved from a second altruistic (or subjugated) embryo.

Further circumstantial evidence supporting the supernumerary embryo hypothesis comes from a comparative analysis of endosperm ontogeny. In many flowering plants, including the model plant *Arabidopsis thaliana*, endosperm development is initially free-nuclear (i.e., no cell walls are laid during the first nuclear divisions); whereas embryo development is always cellular (cell wall formation follows nuclear divisions). However, in

basal flowering plants this distinction is not so clear: in a similar way to the embryo, endosperm development is initially cellular and forms two distinct chalazal and mycropylar domains (18, 19). Interestingly, embryo development in most gymnosperms is itself free-nuclear (20).

The origin of an endosperm and the emancipation of dads

The process of double fertilization and the resulting production of a biparental endosperm is a hallmark of sexual reproduction in flowering plants. The evolution of an endosperm drastically changed the regulation of embryo development from being an exclusive maternal affair (controlled through the female gametophyte and sporophyte) to allowing a substantial degree of paternal control. Different hypotheses have been advanced to explain the advantages conferred by an endosperm (reviewed in 21). Higher levels of heterozygosity and ploidy in the endosperm were initially suggested to allow a more vigorous role in embryo nutrition than the female gametophytes of gymnosperms could provide. Another set of theories viewed the rise of the endosperm as the outcome of a conflict of interests between mother, fathers and offspring in the allocation of resources from the maternal sporophyte [kin conflict (22–24)]. These conflicts arise because each individual offspring competes with its siblings for resources from the maternal sporophyte. Each mother is equally genetically related to all its siblings, so it is in its interest to provide equal levels of nourishment to all its embryos. By contrast, the offspring of a father is typically in direct competition with offspring from other fathers. It is in the interest of the father to maximize the allocation of resources to its own offspring, at the expense of other progeny from the same mother. Therefore, fathers are predicted to try to maximize nutrient allocation and the growth of seeds, whereas mothers are predicted to equitably allocate resources and constrain seed growth. In earlier seed plants (and in extant gymnosperms), the only option for male progenitors to increase the success of their offspring was to improve the fitness of the male gametophytes, gametes and embryos. These were often in direct competition with those of other males, because in gymnosperms multiple embryos develop within each female gametophyte until only one embryo becomes dominant (25). With the evolution of a biparental nourishing tissue, angiosperm fathers could have a more direct role in the nourishing and development of their offspring.

The resurgence of moms: ovule development and endosperm genetics

Soon after the evolution of a biparental endosperm, some lineages of early flowering plants doubled the ploidy of the central cell (the maternal precursor of the endosperm). This resulted in a doubling of the maternal chromosome contribution to the endosperm, allowing mothers to regain privileged control over embryo development.

Endosperm and embryo sac genetics are intimately related. There is a high diversity in embryo sacs types, particularly in the ploidy and genetic composition of central cells, among different flowering plants (8). This diversity can be easily understood in the context of embryo sac ontogenesis (Figure 2). Embryo sacs derive from haploid megaspores that are produced through meiosis from a diploid megaspore mother cell. In most seed plants, three of the four megaspores degenerate. The surviving one (the functional megaspore, situated at the chalazal end) undergoes three free-nuclear divisions, forming eight nuclei within a syncytium. The syncytium later cellularizes to give rise to two synergids and an egg cell at the micropylar end, a central cell with two nuclei, and three antipodals at the chalazal end. This type of monosporic seven-celled/eight-nucleate embryo sac, known as the Polygonum-type, gives rise to a triploid endosperm after fertilization; because the endosperm is derived from a central cell with two nuclei it has a 2:1 ratio of maternal to paternal chromosomal contributions. However, embryo sacs can be derived not only from a single functional megaspore (monosporic type), but also from two megaspores (bisporic type) or even from all four meiotic products (tetrasporic type). Further variation occurs in the number and spatial organization of mitotic nuclei in the mature female gametophyte. A comparative analysis in different types of flowering plants suggests that embryo sacs are modular structures based on repetitions of developmental motifs (26, 27). A basic module is a quartet, formed by a nucleus that undergoes two free-nuclear mitoses to yield four nuclei (Figure 2). Three of these nuclei are partitioned into a pole of the embryo sac while the fourth contributes to the common cytoplasm of the central cell. In Polygonum-type embryo sacs one quartet forms the micropylar egg apparatus (egg cell plus two synergids) and one of the polar nucleus of the central cell; the second quartet corresponds to the three antipodals plus the second polar nucleus of the central cell.

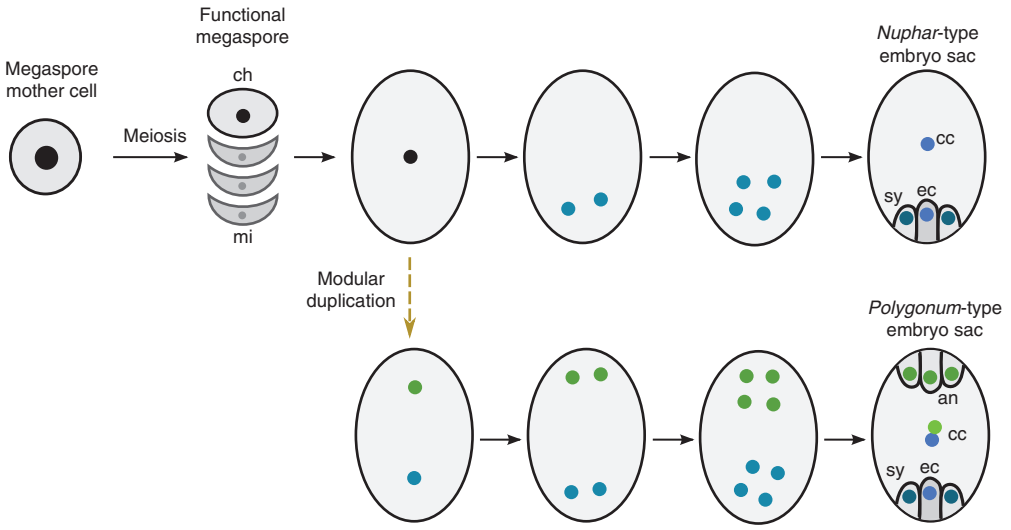

Figure 2 Development of Polygonum-type and Nuphar-type embryo sacs, showing how a duplication of a quartet module could give rise to the doubling in ploidy of the central cell.
ch, chalazal pole; mi, micropylar pole; cc, central cell; sy, synergid; ec, egg cell; an, antipodals.

Unlike the vast majority of angiosperms, the basal flowering plant lineages Nymphaeales and Austrobaileyales have a single basic module that gives rise to a monosporic four-celled/four-nucleate embryo sac (Nuphar-type) (26). The central cell of these embryo sacs have a single nucleus, and therefore gives rise to diploid endosperms. The single quartet module giving rise to the four-celled embryo sac and diploid endosperm of the Nymphaeales and Austrobaileyales is possibly the ancestral condition, present in the first flowering plants. If this hypothesis is correct, a duplication of this module appears to have occurred independently at least twice: once in the *Amborella* lineage (the most basal of living flowering plants, and which has eight-celled embryo sacs) (28) and also in the common ancestor of monocots, eudicots and magnoliids (26, 27). Later, in different groups of flowering plants, further duplications of the basic quartet module gave rise to bisporic embryo sacs (which also originate triploid endosperms), and tetrasporic embryo sacs (which originate triploid, pentaploid, nonaploid or decapentaploid endosperms) (8, 29).

After the evolution of triploid endosperms there were practically no reversals to diploid endosperms or to a strategy of embryo nourishment directly through the female gametophyte [one possible exception are the Nymphaeales, where the diploid endosperm is reduced and the nourishment of the embryo is provided by a perisperm derived from the maternal sporophytic nucellus (30, 31)]. An endosperm with an asymmetric contribution of parental genomes became an integral part of the embryogenesis process of flowering plants.

Paternal and maternal contributions are not equivalent

A correct balance of parental genome contributions on the endosperm is crucial for correct endosperm development. When individuals of different ploidies are crossed, the endosperm often fails to develop properly. This phenomenon, called triploid block, was initially puzzling to plant breeders because while interploidy crosses failed, crosses between tetraploids or hexaploids (resulting in progeny with various levels of ploidy) were viable. Triploid block was eventually explained as a requirement for a correct balance of 2:1 maternal to paternal genomes in the endosperm (32–35).

If the paternal genome contribution is in excess (e.g., when crossing a diploid female with a tetraploid male, resulting in a 2:2 maternal to paternal ratio of chromosomes in the endosperm), the endosperm typically overproliferates because of an accelerated rate of mitotic division and delayed cellularization (36, 37), associated with an altered timing of cell cycle progression (38). In cases where viable seeds can be produced from paternal excess crosses, seeds are larger and heavier than normal seeds. Conversely, if the maternal contribution is in excess (e.g., in the reciprocal cross of a tetraploid female with a diploid male, resulting in a 4:1 maternal:paternal ratio in the endosperm), the endosperm exhibits reduced mitotic divisions and precocious cellularization, resulting in seed abortion or the production of smaller and lighter seeds.

The opposite effects of reciprocal interploidy crosses on endosperm development demonstrate that the parental

genomes are not equivalent and that mothers and fathers have opposite impacts on the growth of their offspring. Interestingly, many interspecific crosses result in reciprocal endosperm failure phenotypes that resemble the failure of interploidy crosses (reviewed in 39–41). Increasing the ploidy of one of the parents can sometimes rescue otherwise unsuccessful crosses (40, 42). This suggests that the common cause of endosperm failure following interspecific and interploidy crosses is a disruption of the balance of maternal and paternal gene expression (39, 43).

A simplistic interpretation of the effects of interploidy crosses on seed development is that the maternal genome restricts endosperm development, while the paternal genome promotes it, thereby increasing the potential for the formation of larger and better nourished embryos. Maternal control of seed size can also be exerted directly through the (maternal) seed coat. In contrast, endosperm vigor is controlled by both maternal and paternal genomes. The transition from the syncytial phase (during which endosperm nuclei proliferate through multiple rounds of mitosis without cytokinesis) to the cellularized phase (during which cell walls are formed) is an important developmental transition during seed development (44–46). Accordingly, the timing of endosperm cellularization is particularly sensitive to the balance of parental genomes, with maternal excess crosses typically promoting premature endosperm cellularization and paternal excess promoting a delaying of endosperm cellularization (47). Nevertheless, the maternal nature of seed coats and the higher genome dosage in the endosperm allows the maternal side to have a strong control on seed size. In *Arabidopsis*, seed size has been shown to be predominantly determined by the maternal genotype, while the paternal genotype explains only around 10% of the variation (48).

Imprinting

The balance of paternal and maternal gene expression can be regulated in two main ways: by the relative ploidy of the central cell (discussed above) and by the differential expression of alleles from each parent. One extreme case of differential allelic expression is imprinting, where only one of the parental alleles is expressed. The maize *R* gene was the first imprinted locus to be discovered (49) and today several imprinted genes are known in flowering plants and mammals (reviewed in 50–52). The evolutionary origins of imprinting are not well understood, but a theory proposed by David Haig and Mark Westoby (the parental-conflict theory of the evolution of genomic imprinting) offers a popular (but sometimes misinterpreted) explanation for some types of imprinting (39, 53). The assumption is that in a maternal plant with progeny from different fathers, paternally-derived genes in any given seed have an interest in maximizing the success of that seed (at the expense of seeds from other fathers), whereas maternally-derived genes have an interest in also promoting the success of other seeds. This hypothesis predicts that this conflict favors the expression of maternal genes that silence paternal genes that would otherwise increase nutrient acquisition demands; this eventually gives rise to mono-allelic expression (imprinting).

It is important to highlight that this theory does not explain all types of imprinting, and that imprinting can explain some, but not all, parent-of-origin effects. Parent-of-origin effects are perhaps better explained by the differential dosage hypothesis that involve differential biallelic expression (and not just monoallellic expression) of dosage-sensitive regulators (43). In plants, most imprinted genes are expressed in the endosperm, but there are also some genes imprinted in the embryo (54, 55). Interestingly, early embryo development may be predominantly controlled by maternal transcripts, although different studies have come to different conclusions (56–60).

In the next section the two main epigenetic mechanisms that control imprinting will be discussed: DNA methylation (particularly CG methylation) and histone methylation, particularly repressive H3K27me3 marks catalyzed by polycomb group proteins.

DNA methylation

In *Arabidopsis*, the DNA methyltransferase MET1 is responsible for the maintenance of genome-wide CpG methylation (61). Crosses between a hypomethylated *met1* mutant pollen donor and a normal seed parent mimic the phenotype of a maternal excess cross between a diploid father and a tetraploid mother, resulting in small seeds and in a reduction of endosperm mitotic divisions (62, 63). This suggests that parent-of origin effects require DNA methylation. One explanation is that the release of repressive methylation marks from paternally silenced genes originates extra transcription of otherwise maternal-specific genes, originating a maternal excess phenotype. The reciprocal cross of a hypomethylated *met1* seed parent with normal pollen mimics the phenotype of a paternal excess cross (40, 62, 63), although this appears to rather be caused by a sporophytic effect in the cell proliferation in the seed integuments, and not by a gametophytic effect (64). The maintenance of *MET1* CpG

methylation in the gametophytic phase has been shown to be essential for the inheritance of epigenetic marks (65). Together, these observations suggest that CpG methylation mediated by *MET1* plays an important role in the maternal control of seed development by restricting cell proliferation in the sporophytic integuments and by restricting the expression of paternal genes in the endosperm.

The imprinting of several genes is mediated by DNA methylation. In *Arabidopsis*, DNA methylation is predominantly controlled by the antagonist action of the DNA methyltransferase MET1 and the DNA demethylase demeter (DME). *DME* is expressed in the central cell of the embryo sac before fertilization but not in pollen sperm cells (66, 67), whereas *MET1* is strongly expressed in sperm cells but repressed in the central cell by the retinoblastoma pathway (68–70) (Figure 3). As a consequence, the maternal genomes that are contributed to the endosperm are demethylated relative to the paternal genomes and embryo genomes (71–73). The DNA demethylation that occurs in the central cell is partially responsible for the maternal expression of imprinted genes such as *MEA*, *FWA*, *FIS2* in *Arabidopsis* and *FIE2* in maize (74–78).

Polycomb and histone methylation

In addition to DNA methylation, imprinting in both plants and animals is also regulated by histone methylation (50, 52, 79, 80). The Polycomb Repressive Complex 2 (PRC2) catalyzes trimethylation of histone H3 at lysine 27 (H3K27me3), a repressive mark associated with gene silencing. The four core PRC2 subunits are highly conserved between plants and animals, but in plants each subunit is usually represented by a small gene family (81, 82). In *Arabidopsis*, the Fertilization Independent Seed (FIS) PRC2 is active during seed development and is comprised of the SET domain histone methyltransferase MEDEA (MEA), the zinc finger protein Fertilization Independent Seed2 (FIS2), and the WD40 domain proteins Fertilization Independent Endosperm (FIE) and Multicopy Suppressor of IRA1 (MSI1). Plants with loss-of-function mutations in any of these PRC2 subunits (*fis* mutants) develop a uniparental endosperm in the absence of fertilization. In addition, maternally inherited *fis* mutations cause defects in endosperm proliferation and embryo growth that are reminiscent of the effects caused by paternal excess crosses (45, 83–89).

Members of the FIS-PRC2 complex are themselves imprinted in different flowering plants. In *Arabidopsis*, the paternal alleles of *MEA*, *FIS2* and *FIE* are neither expressed nor required for endosperm development (90–92). Interestingly, the maternal allele of *MEA* regulates the silencing of its paternal allele via H3K27me3 (75, 93, 94). Homologs of *FIE* are imprinted in the endosperm of maize and rice (95, 96) and one homolog of *MEA* is imprinted in the endosperm of maize (97). This suggests that the imprinting of at least one endosperm PRC2 element may be required for correct endosperm development across different species. In the basal eudicot *Aquilegia* there is no evidence for imprinting of *MEA* homologs (98), although it remains to be tested whether other PRC2 components are imprinted.

The relationship between paternal dosage effects, the imprinting of FIS-PRC2 components and the function of FIS-PRC2 in imprinting is multi-directional. Not only do loss-of-function *fis* mutants 'paternalize' the seeds, but paternal excess crosses also deregulate imprinting of *FIS2* and *MEA* (99, 100). The transcriptome of seeds with paternal excess is similar to the transcriptome of *mea* and *fis2* seeds (99, 101). Increasing the maternal dosage (using a mutant that forms unreduced gametes) restores correct cellularization of the endosperm and partially rescues *mea* and *fis2* seeds (102). Together, this suggests that the FIS-PRC2 acts as a maternal regulator of seed development and, as a consequence, losing FIS-PRC2 function 'paternalizes' seeds. Restoring the parental balance in *fis* mutants by increasing maternal ploidy restores the viability of the cross. Reciprocally, the defects caused by a paternal excess cross can be reversed by artificially increasing the expression of *MEA* (99).

Strikingly, the endosperm failure phenotype of *fis* mutants can be rescued if the paternal genome is entirely absent. Pollen derived from *cdka; 1 Arabidopsis* mutants can successfully fertilize only one of the female gametes (103, 104). If a sperm cell from *cdka; 1* pollen fertilizes the egg cell of a *fis* mutant, diploid endosperm development can progress and produce viable, but small seeds (105).

Pollen hypomethylation derived from a knockdown of *met1* can partially suppress seed abortion caused by loss of *mea*, *fie* and *fis2* mutants (92), further supporting the idea that pollen hypomethylation has a maternalizing effect and *fis* mutants have a paternalizing effect in endosperm. Nevertheless, a functional paternal *FIE* allele is required for *fis* seed rescue by *met1* (106), suggesting that the effects of hypomethylation can counteract FIS-PRC2 but not other PRC2 complexes (which all require a functional FIE).

Many loci that are targeted by H3K27me3 in the endosperm have reduced DNA methylation levels in the

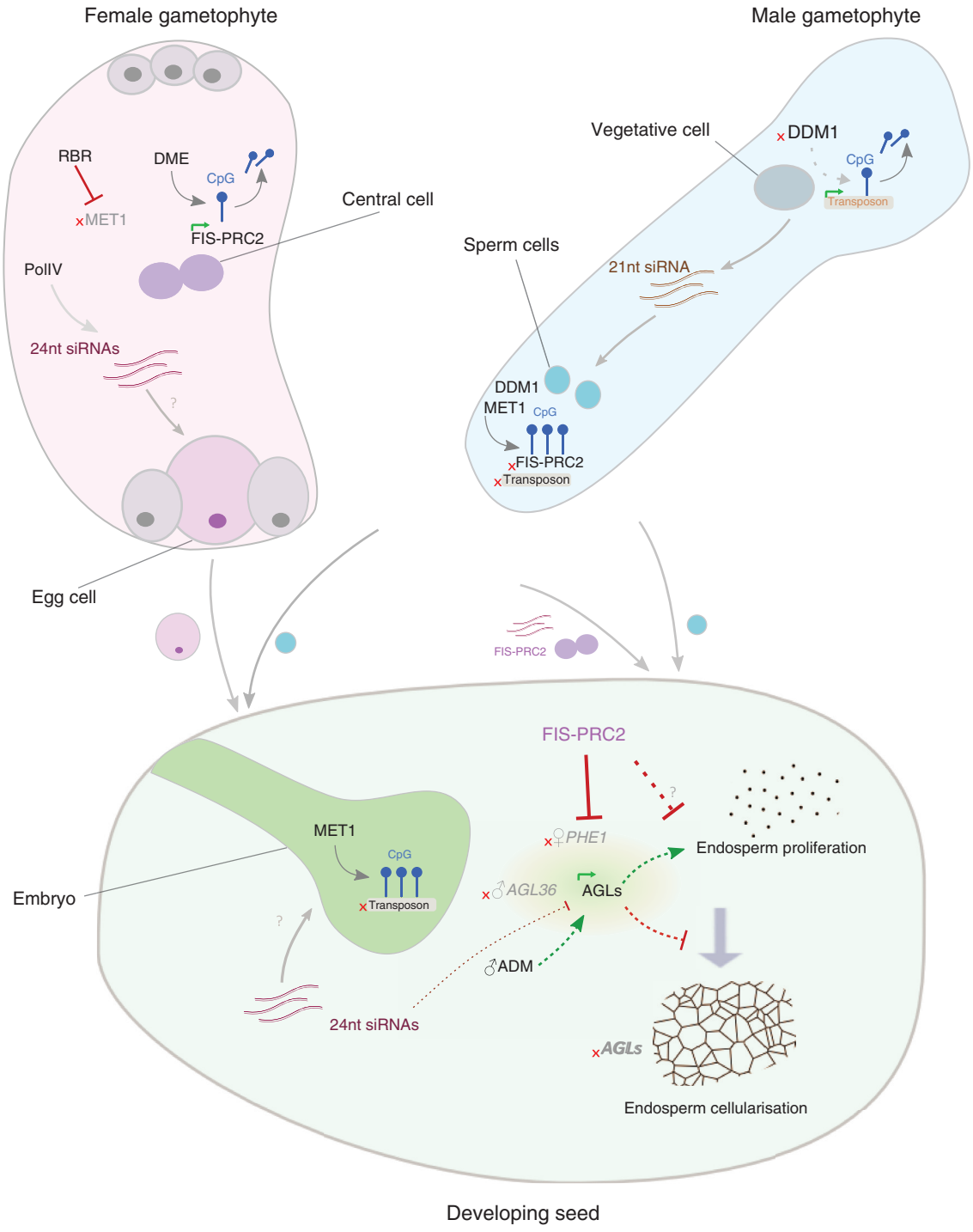

Figure 3 Highly simplified diagram showing the genetic and epigenetic mechanisms that balance paternal and maternal information during gametogenesis and seed development.

endosperm compared with vegetative tissues. This suggests that loss of DNA methylation allows loci to be targeted by H3K27me3 marks in the endosperm (107). Interestingly, DNA methylation in a region downstream of the imprinted gene *PHE1* is required to activate paternal expression. It is possible that loss of DNA methylation is required for allowing access to FIS-PRC2 proteins that mediate the stable repression of *PHE1* allele (108). This is in contrast to many other loci, in which DNA methylation is required for silencing.

Mutations in *MEA*, *FIS2*, *FIE* and *MSI1* result in autonomous endosperm development in *Arabidopsis* (83, 85–89), suggesting that FIS-PRC2 plays a central role in restricting endosperm development in the absence of fertilization. Interestingly, loss-of-function mutants of PRC2 components in the moss *Physcomitrella patens* develop sporophyte-like bodies directly on the gametophyte, without the formation of gametes or fertilization (109, 110). This phenomenon, known as apogamy, is common in diverse groups of bryophytes and ferns (111). It is tempting to speculate that the development of an endosperm from a central cell in the absence of fertilization is homologous to the apogamy process of basal plants. If this is the case, it suggests that the requirement for PRC2 function to prevent apogamy is an ancient mechanism in plants, and was later recruited to prevent autonomous endosperm development in flowering plants.

MADS transcription factors and the timing of endosperm cellularization

The failure of endosperm development following incompatible interspecific/interploidy crosses and in PRC2 mutants is mediated by several type-1 MADS box proteins. Many of these transcription factors have recently been identified as central regulators of plant reproductive development, from patterning the female gametophyte to controlling the timing of endosperm cellularization (112).

PHERES1 (*PHE1*) is maternally silenced by H3K27me3 marks deposited by the maternally active FIS-PRC2, so that only the paternal allele is expressed (113–115). The imprinting of *PHE1* is lost in *fis* mutants (114) and following incompatible crosses between *A. thaliana* and *A. arenosa* (116). The *mea* endosperm over-proliferation phenotype is partially mediated by PHE1, as reducing *PHE1* expression can reduce *mea* seed abortion (113). Another key type-1 MADS box gene regulating endosperm development is *AGL62*. *AGL62* is expressed during the proliferating phase of endosperm development and drops just before cellularization because of FIS-PRC2 activity (46, 117). *agl62* mutants undergo precocious endosperm cellularization, suggesting that AGL62 suppresses cellularization during syncytial development (117). Other *AGLs* (*PHE2*, *AGL35*, *AGL36*, *AGL40*, and *AGL90*) are also upregulated following incompatible crosses between *A. thaliana* and *A. arenosa* that resemble paternal excess crosses (118), and several *AGL* genes are downregulated in uniparental endosperm (119). Conversely, in maternal excess crosses these *AGL* genes are downregulated (102). In *A. thaliana*, *AGL36* has been shown to be expressed exclusively from the maternal allele (119).

These results suggest that *AGL* genes act downstream of FIS-PRC2 to positively regulate endosperm proliferation and/or negatively regulate endosperm cellularization (Figure 3). Supporting this hypothesis, inactivating *PHE1*, *AGL62* or *AGL90* reduces endosperm over-proliferation and reduces seed failure in *fis* mutants and in interspecific crosses between *A. thaliana* and *A. arenosa* (46, 113, 118). This hypothesis suggests that the defects in endosperm development associated with loss of FIS-PRC2 function, parental genome unbalance and interspecific crosses are at least partially caused by misexpression of *AGL* genes. Indeed, the role of *AGL* genes in controlling endosperm development appears to be quite ancient. In rice, at least one type-1 MADS box gene is maternally imprinted. Following interspecific crosses imprinting is lost and other rice MADS box genes also become deregulated (41). Interestingly, a genomic widespread disruption of imprinting has been reported in interspecific crosses in rodents (120).

MADS proteins are not the only mediators of the balance of parental genomes and FIS-PRC2 activity. Loss-of-function of a paternally expressed J-domain molecular chaperone, ADMETOS, can partially rescue the seeds derived from paternal excess crosses and from a *mea* mutant (121).

The hidden roles of small RNAs

In the last years small RNAs were shown to play a central role in regulating the epigenetic state of the gametes and of the endosperm. In *Arabidopsis*, the production of 24nt PolIV-derived siRNAs (p4-siRNAs) in the endosperm is under strict maternal control (122). The maternal p4-siRNA population accumulates at high levels in the central cell and during endosperm development, suggesting that these small RNAs may move to the egg and embryo to reinforce silencing of TE through non-CG methylation (Figure 3) (123–125). This mechanism is still very unclear, and the 24nt RNA-directed DNA methylation (RdDM) mechanism was conversely suggested to be repressed in the female gametes, leading to activation of the maternal alleles and causing DME-independent imprinted expression in some loci (126).

A similar mechanism whereby an accessory cell loses its genome integrity in order to reinforce silencing in the gametes was proposed to occur in pollen. The chromatin remodeling ATPase *DECREASE IN DNA METHYLATION 1* (*DDM1*) is a central regulator of TE activity in *Arabidopsis*.

DDM1 accumulates in pollen sperm cells, but not in the pollen vegetative nucleus (127). The absence of DDM1 protein in the pollen vegetative nucleus causes a loss of heterochromatin and a massive activation of TE, which leads to the production of 21nt siRNAs. These siRNAs are then exported to the sperm nuclei to reinforce TE silencing (127).

The changes in the chromatin status of the vegetative nucleus of pollen and of endosperm nuclei (127–129), associated with the massive maternal and paternal production of 24nt and 21nt siRNAs are evidence of the existence of large epigenetic changes during gametogenesis and embryogenesis. This led to the proposal that a 'genome shock' causing TE to escape methylation is partially responsible for hybrid incompatibility (hybrid dysgenesis) following interspecific and interploidy crosses (130). However, it is not obvious how TE activation alone could lead to the characteristic over-proliferation and/or precocious cellularization phenotypes of parentally unbalanced endosperms. Furthermore, *nrpd1a* mutants, which are impaired in the production of p4-siRNAs, do not show an obvious difference in endosperm growth (122). Nevertheless, lack of p4-siRNAs induces an upregulation of *AGL* genes (131). In other words, the loss of the maternal p4-siRNAs paternalizes the endosperm, at a transcriptomic level. One possible explanation for the lack of a requirement for p4-siRNA production for normal seed development *Arabidopsis* may be that these mechanisms are relaxed in a predominantly inbreeding species. Interestingly, the 24nt p4-siRNA mechanism may have evolved with the flowering plants (132).

In incompatible interspecific crosses between *A. thaliana* and *A. arenosa*, *ATHILA*, a major *Arabidopsis* retrotransposon that is typically silenced, becomes expressed from the paternal alleles (116). This may be because maternal siRNAs are unable to repress the paternally-derived retrotransposons. However, other transposons than *ATHILA* do not appear to become activated in the *A. thaliana* x *A. arenosa* cross (116, 133), suggesting that retrotransposon activation is not a general consequence of interspecific crosses.

Seed coat: an additional layer of maternal influence

Seeds are multi-generational structures enveloped by a seed coat of maternal sporophytic origin. The seed coat offers an effective means through which mothers can regulate seed development, particularly by determining seed size. In *Arabidopsis*, several maternal sporophytic mutations that affect seed size and shape through integument (testa) development have been isolated (134–138). One important maternal sporophytic regulator of seed size in *Arabidopsis* is the WRKY transcription factor TRANSPARENT TESTA GLABRA2 (TTG2). The endosperm of seeds developing from maternal homozygous *ttg2*/*ttg2* mutants cellularize precociously and seeds are small; however, maternal heterozygous *ttg2/TTG2* mutants generate normal seeds, suggesting that *TTG2* controls seed development as a maternal seed coat sporophytic factor, possibly by controlling integument cell elongation (138). Natural allelic variation at the *TTG2* locus is also responsible for variation in the tolerance to interploidy crosses in *Arabidopsis*, and *ttg2* mutants can reduce seed lethality in paternal excess and in interspecific crosses (133, 139).

Integument growth can nevertheless be regulated by the rate of endosperm growth. A putative pathway formed by the VQ motif protein HAIKU1, the leucine-rich kinase HAIKU2, the WRKY transcription factor MINI3 and the cytokinin oxidase CKX2 regulates endosperm proliferation; mutations in these genes cause reduced endosperm growth and precocious cellularization, and this negatively impacts on integument elongation (140–143). The initiation of seed coat development from ovule integuments upon fertilization is controlled by signaling from the endosperm. *fie* and *msi1* mutant seeds can develop an endosperm in the absence of fertilization, and this autonomous endosperm development is sufficient to initiate seed coat development (87, 144, 145). Nevertheless, it has also been proposed that a correct signal initiating seed coat development requires a sexual, fertilized endosperm (145). Other non-FIS polycomb group proteins such as VERNALIZATION2, EMBRYONIC FLOWER2 and SWINGER act in the sporophytic maternal integuments to restrict autonomous seed development (145).

Outlook

The last 15 years were a particularly productive period in which we greatly increased our understanding of how seeds evolved and how they develop. Sophisticated phylogenetic analysis and detailed comparative analyses at the base of the angiosperm family gave us a glimpse into some of the steps that resulted in the appearance of an endosperm. However, it is still far from clear how the transition from the polyembryonic seeds of gymnosperms gave rise to double fertilization and the endosperm in angiosperms, and how the diploid endosperm of early

angiosperms gave rise to the triploid endosperm of *Amborella* and most angiosperm lineages. Over the next years it is expected that further comparative analyses will help to clarify this. In addition, the increasing power and availability of genomics and transcriptomics should also help us to understand how seed development in basal angiosperms (and indirectly in early angiosperms), relates to seed development in more derived lineages such as *A. thaliana* and the grasses.

Much of what we learned from seed and endosperm development in these 15 years was made possible by the many resources and tools available for *Arabidopsis*. These are continuously expanding, and large-scale analyses made possible by next-generation sequencing are already painting a detailed picture of the transcriptomic dynamics that occur throughout seed development. Omics approaches will become more recurrent in the future, and the massive amount of data accumulated will require the adoption of computational and mathematical modeling.

Conversely, some of the traits that make *A. thaliana* such a good tool for experimental biologists, particularly its inbred character, also make it a poor choice for studying parental conflicts and imprinting, as these are less intense in self-pollinating plants than in outcrossers (146). *A. thaliana* atypically tolerates crosses where the genome contribution of one of the progenitors is doubled (36), while *MEA* has been shown to evolve faster in the outcrosser *A. lyrata* than in *A. thaliana* (147, 148). Endosperm and epigenetics research in rice and maize have provided an invaluable complement to *Arabidopsis* research, but parental-conflict in these crop species can also be predicted to be distorted by the very strong artificial selection that these species underwent over the last thousands of years. A strategic sampling and study of non-model species will help us to have a clearer picture of how the interaction of paternal and maternal genomes controls seed development.

Highlights

- Embryos of land plants were progressively embedded within parental tissues, a process that culminated in the evolution of seeds and the endosperm.
- The evolution of an endosperm allowed paternal genes to participate in seed development, setting the conditions for the manifestation of conflicts of interest between maternal and parental genomes.
- A correct balance of maternal and paternal gene dosage in the endosperm is required for correct seed development.
- The balance of parental gene dosages in the endosperm can be adjusted by changes in the ploidy of the central cell or by differential allelic expression (e.g., imprinting).
- MADS transcription factors are central regulators of the transition to endosperm cellularization.
- Similar mechanisms underlie the endosperm development failure phenotypes caused by PRC2 mutants, interploidy and interspecific crosses.
- The role of PRC2 complex in restricting autonomous endosperm development and over-proliferation may have evolved from a more ancient role in restricting apogamy.
- Small RNAs undergo massive de-repression in the endosperm and in the vegetative nucleus.

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Dr. Nuno Pires works at the Institute of Plant Biology of the University of Zurich. He did his PhD in the group of Prof. Liam Dolan (John Innes Centre and University of Oxford), where he studied the evolution of gene regulatory networks in land plants. He is currently a postdoctoral researcher in the group of Prof. Ueli Grossniklaus, where he studies natural variation and the genetics of endosperm development in *Arabidopsis thaliana*.